Chapter 53

Plant Growth Promotion by Microbes

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ABBREVIATIONS

AHL, *N*-Acyl homoserine lactone; AMF, Arbuscular mycorrhizal fungi; LCO, Lipochitooligosaccharide; MHB, Mycorrhiza helper bacteria; PGP, Plant growth promotion; SL, Strigolactone; TTSS, Type three secretion system.

53.1 INTRODUCTION

The world's population is assumed to increase from 7 billion now to 8.3 billion in 2025. The world will need 70–100% more food by 2050 (Godfray et al., 2010). Therefore, the production of cereals, especially wheat, rice, and maize, which accounts for half of human calorie intake, has to be increased. Currently, plant growth is enhanced by the input of chemicals which act as plant growth regulators (using a hormonal mechanism) and as nutrients. Of the nutrients added to the soil, nitrogen and phosphorus are the major ones. They are, together with potassium, applied as chemical fertilizers to improve grain yield. According to Roberts (2009), the present global annual use of chemical nitrogen, phosphorus, and potash fertilizer is 130, 40, and 35 million tonnes, respectively.

The high input of chemicals raises a number of concerns such as water contamination leading to eutrophication and health risks for humans. Moreover, it results in soil degradation and loss of biodiversity. In this chapter, we will describe beneficial microbes which can act as environmentally friendly alternatives for agrochemicals. Their application will increase the sustainability of agriculture.

We subdivide these beneficial microbes in the following groups. (A) General plant growth promoters. These microbes stimulate plant growth through a variety of mechanisms or by one or more unknown mechanisms. (B) Microbial fertilizers for specific nutrients, the most important ones being N, P, and Fe^{3+}. (C) Microbial plant growth regulators. These secrete hormones or hormone-like substances which stimulate plant growth in extremely low concentrations. This subdivision is not perfect as one microbe can combine several mechanisms.

The major global nutrition processes are illustrated in the figures, whereas, the plant growth promotion (PGP) traits of some species are listed in Table 53.1.

53.2 GENERAL MICROBIAL PLANT GROWTH PROMOTERS

Some microbes and molecules have a general plant growth-promoting effect. They can stimulate, for

Molecular Microbial Ecology of the Rhizosphere, Volume 2, First Edition. Edited by Frans J. de Bruijn.

Table 53.1 PGP microbes and their relevant PGP traits[a]

Trait	Reference
(a) Arbuscular Mycorrhizal Fungi	
Function as extension of the root system; hyphae reach sites where roots cannot come; not host specific	Chapter 43
AMF branching and contact formation with roots stimulated by SLs (strigolactones)	Chapters 33–35
AMF secrete Myc factors which stimulate root growth and branching	Maillet et al. (2011)
Uptake of water, P, Zn, Cu, and other nutrients	Clark and Zeto (2000)
Improvement of soil structure	Smith and Read (2008)
Protection against (a)biotic stresses	Smith and Read (2008)
Mycorrhiza helper bacteria (MHB) promote pre-symbiotic survival and fungal growth	Frey-Klett et al. (2007); Chapter 49
(b) Trichoderma	
Can act as endophyte; increases uptake of water and nutrients; increases solubilization of soil nutrients; increase of nitrogen use efficiency; enhancement of plant vigor; enhanced growth and development of roots and above-ground plant parts; increases root hair formation; causes deeper rooting; improved photosynthetic efficiency; uses exudate sucrose; uses hydrophobins and expansin-like proteins for attachment to roots	Harman (2006), Lorito et al. (2010), Shoresh et al. (2010), Hermosa et al. (2012)
Degrades phenolic compounds secreted by plants	Ruocco et al. (2009)
Produces auxin	Contreras-Cornejo et al. (2009)
Accelerates seed germination	Mastouri et al. (2010)
Increase of plant resistance, especially under suboptimal growth conditions	Lorito et al. (2010)
Amelioration of abiotic stress; alleviation of physiological stresses, e.g., seed aging	Mastouri et al. (2010), Shoresh et al. (2010)
The secondary metabolite harzianic acid promotes plant growth	Vinale et al. (2009)
(c) Bacillus	
N_2-fixer	Borriss (2011); see Chapter 83
Phosphate solubilizer	Rodriguez et al. (2006), Borriss (2011)
Release of Pi from phytate	Idriss et al. (2002)
Potassium solubilizer	Wu et al. (2005)
(d) Pseudomonas	
Associative N-fixer	Dobbelaere et al. (2003)
Phosphate solubilizer	Rodriguez et al. (2006); see Chapter 58
Siderophore producer	Lemanceau et al. (2009); see Chapter 113
Auxin producer	Kamilova et al. (2006)
Cytokine producer	García de Salome et al. (2001)
ACC deaminase producer	Glick et al. (2007a)

[a] Note that not all strains of the mentioned species have the listed traits and that all listed traits are not present in a single strain.

example, plant establishment and enhance plant vigor. These are discussed in this section. Other microbes have a more specific effect for a certain nutrient. These are discussed in Section 53.3.

53.2.1 Arbuscular Mycorrhizal Fungi (AMF)

Approximately 90% of land plants live in symbiosis with AMF (see Chapter 43; see Fig. 53.1 and Table 53.1a). AMF are not host specific. Combinations of AMF and plant roots can form enormous underground networks. Since exudates from fungal hyphae solubilize more P than root exudates alone, it was suggested that mycorrhizae contribute to increase of P-uptake through P-solubilization. AMF can enhance plant establishment and increase water and nutrient uptake, especially of P, Zn, and Cu (Clark and Zeto, 2000; see Fig. 53.1; Table 53.1a). AMF also protect plants against biotic and abiotic stresses and can improve soil structure (Smith and Read, 2008). Since AMF perform similar functions as roots, they functionally extend the root system. Therefore,

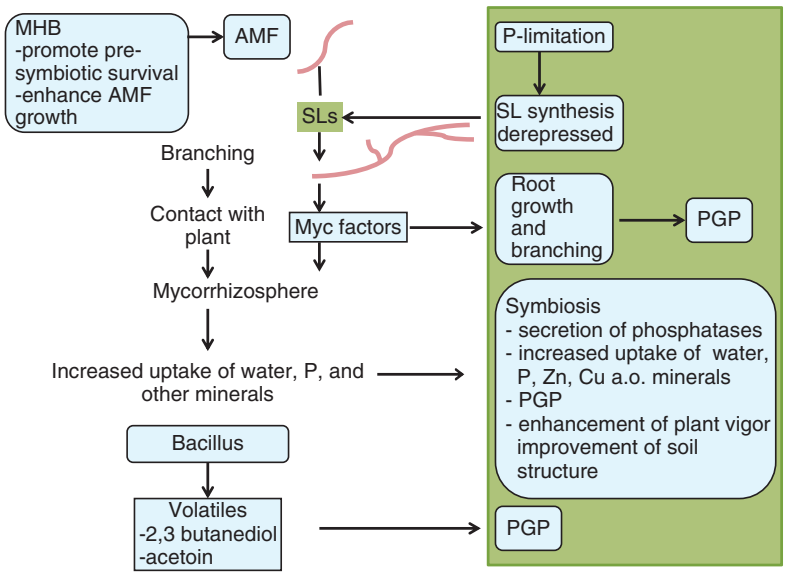

Figure 53.1 Role of Arbuscular Mycorrhizal Fungi AMF in PGP. For explanation, see text and Table 53.1a. Colors: green, plant; red, microbes; blue, processes.

the area around roots with attached AMF is called the *mycorrhizosphere*. Because of their smaller diameter, the fungal hyphae are able to reach places where roots cannot penetrate. AMFs are also beneficial for soil structure because they cause aggregate formation.

Strigolactones (SLs), the recently discovered class of shoot-branching hormones, are involved in early stages of the plant–AMF interaction (See Fig. 53.1; Chapters 33, 34).

They are present in root exudates of both mono- and dicotyledonous plants. Their synthesis is upregulated by phosphate limitation. SLs from root exudate cause branching of neighboring AMF spores, thereby, increasing their chances to encounter a plant root. SLs also influence auxin transport. In principle, SLs or some of their analogs have the potential to be used for weed control: they are able to induce germination of spores of the weed *Striga*, which causes massive crop losses of cereals in developing countries. If this induction takes place in the absence of crop plants, the *Striga* will die (see Schachtschabel and Boland, 2009). See also Chapters 33–35.

AMF produce diffusible symbiotic signals, recently identified as lipochitooligosaccharides (LCOs) and designated as *Myc factors* (See Fig. 53.1; Table 53.1a). They stimulate root growth and branching. It is expected that (derivatives of) these compounds will be used in future agriculture (Maillet et al., 2011; see Chapters 43 and 45).

Some bacteria help AMF (MHBs; Frey-Klett et al., 2007, 2011; see also Chapter 49 and Fig. 53.1). In the case of the *Pseudomonas fluorescens* helper bacterium strain BBc6R8, it was shown that this bacterium promotes the pre-symbiotic survival and growth of the fungus (Deveau et al., 2007).

53.2.2 *Trichoderma*

Although the soil fungus *Trichoderma* is mainly known as a biocontrol agent (see Chapter 54; Harman et al., 2004; Lorito et al., 2010), it has also a large set of direct plant growth-promoting properties (See Table 53.1b). *Trichoderma* is claimed to increase plant resistance under suboptimal growth conditions, to increase nutrient uptake, to increase nitrogen use efficiency, to enhance solubilization of soil nutrients, to enhance growth, vigor, photosynthetic efficiency, development of roots, and above-ground plant parts, to increase root hair formation and to enhance deeper rooting (Harman, 2006; Shoresh et al., 2010; Lorito et al., 2010; See Table 53.1b). Moreover, it can reduce abiotic and physiological stresses. The latter may be because of 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Viterbo et al., 2010). The secondary metabolite harzianic acid has been identified as a plant growth promoter (Vinale et al., 2009; See Table 53.1b). We conclude that *Trichoderma* has properties similar to those of AMF. However, *Trichoderma* has the advantage that it can be grown in pure culture. Products with *Trichoderma* as the active ingredient have been commercialized.

53.3 BIOFERTILIZERS FOR SPECIFIC NUTRIENTS

Plant growth-promoting microbes that fix N_2, solubilize phosphate, and/or produce siderophores are classified as biofertilizers, as they increase the availability of these nutrients to plants (Fuentes-Ramirez and Caballero-Mellado, 2006).

53.3.1 Nitrogen Fixation

N_2 is abundant in the atmosphere, but is unavailable to plants. Plants receive their nitrogen in the form of ammonium (NH_4^+) and nitrate (NO_3^-). Uptake of NO_3^- occurs together with influx of protons, whereas uptake of NH_4^+ occurs together with release of protons. These processes therefore cause alkalinization and acidification of the rhizosphere, respectively, and substantially influence rhizosphere processes.

Conversion of atmospheric N_2 to ammonium is known as the process of biological nitrogen fixation or *diazotrophy*. The ability to fix nitrogen is widespread among prokaryotes with representatives in both bacteria and archaea (Dekas et al., 2009). This reaction is catalyzed by the nitrogenase enzyme complex which in most bacteria contains molybdenum–iron (Mo–Fe) as the cofactor. Some bacteria have an additional nitrogenase containing vanadium (Robson et al., 1986) or only iron (Chisnell et al., 1988). However, the alternative nitrogenases have a lower efficiency of nitrogen fixation compared with the conventional ones (Joerger and Bishop, 1988).

Many diazotrophic bacteria are able to establish a symbiotic relationship with plants. The best-studied symbiotic diazotrophs belong to the gram-negative rhizobia which induce nodules on leguminous plants (Fabales; see Fig. 53.2). The only exception is the genus *Parasponia* which belongs to Rosales but is nodulated by rhizobia (Markmann and Parniske, 2009).

The rhizobium–legume symbiosis is considered to be the major source of fixed nitrogen. It has been estimated that this symbiosis contributes more than 45 million metric tonnes of N per year to the terrestrial ecosystems (Vance, 2001). The current taxonomy of rhizobia includes 12 genera with more than 90 species (Weir, 2011; see Chapter 44) and is still expanding. The best-known rhizobia are those of the α-subclass of Proteobacteria (*Allorhizobium*, *Azorhizobium*, *Rhizobium*, *Mesorhizobium*, *Ensifer* [former *Sinorhizobium*] and *Bradyrhizobium*; see also Chapter 94). In addition, several β-proteobacteria belonging to *Burkholderia* and *Cupriavidus* have been shown to nodulate plants (Moulin et al., 2001). Rhizobia and other N-fixing bacteria share essential *nod* and *nif* genes encoding nodulation and nitrogen fixation functions, respectively (Zehr and Turner, 2001). These genes are often carried on symbiotic plasmids which are highly transferable (Brom et al., 2004). Moreover, recipient bacteria are able to obtain a symbiotic function after being transformed with these plasmids (Rogel et al., 2001). As this can happen under both laboratory and field conditions, it might partly explain the diversity of root-nodulating bacteria (see Chapter 44).

The symbiosis is initiated by root exudate components, flavonoids, or isoflavonoids, which, after uptake by the bacterium, activate *nod* genes in the bacterium (See Fig. 53.2; see Chapter 51). The bacterial answer in this molecular dialogue is secretion of products encoded by the *nod* genes, the NOD factors. NOD factors are lipo–chitin oligosaccharides differing from each other in the length of the chitin fragment, in the unsaturation of their fatty acyl chain, and in the presence of several molecular decorations. This makes NOD factors major determinants of the host specificity of symbiosis (Spaink et al., 1998; see also Chapter 45; Fig. 53.2). Specific perception of NOD factors by plants results in activation of a set of plant genes leading to the formation of root nodules and entry of bacteria (Geurts and Bisseling, 2002; see Chapter 45). However, certain photosynthetic bradyrhizobia lacking *nod* genes

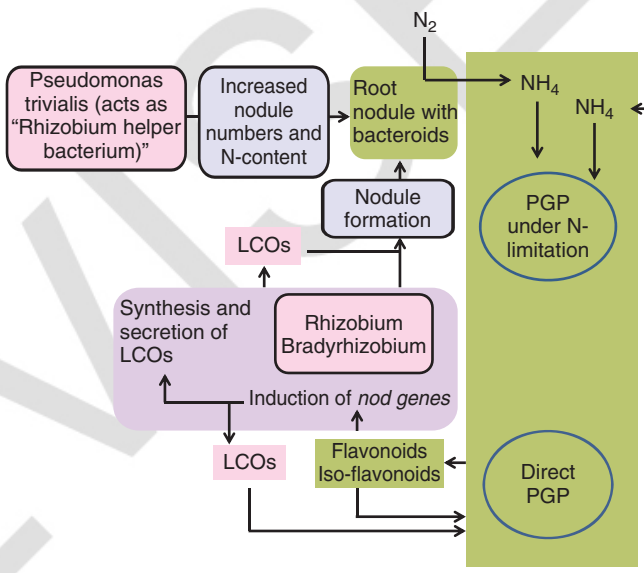

Figure 53.2 Microbial contribution to plant N-nutrition. For explanation, see text. Colors: green, plant; red, microbes; blue, processes.

rely on a different, yet to be characterized strategy for plant signaling (Giraud et al., 2007). *nod* Genes have not been detected in the genome of *Frankia*, a gram-positive bacterium from the family Actinobacteria which nodulate non-leguminous plants belonging to the Rosales, Fagales, and Cucurbitales. These interesting findings represent a promising source for developing nitrogen-fixing cereals.

Rhizobia can interact with other plant-associated bacteria in the rhizosphere. Such a cooperation can have a beneficial effect on plant growth. For example, Egamberdieva et al. (2010) recently showed that co-inoculation of fodder galega with *Rhizobium* and biocontrol pseudomonads improves shoot and root dry matter of the plant. One of these strains, the cellulase-producing *Pseudomonas trivialis* 3Re27 (Scherwinski et al., 2008), significantly increased nodule numbers and nitrogen content of the co-inoculated plant. The authors coined the term "*Rhizobium* helper bacteria" for this biocontrol strain (See Fig. 53.2).

In addition to symbionts, there are also free-living and associative diazotrophs; these include bacteria from a number of genera: *Acetobacter*, *Azoarcus*, *Azospirillum*, *Azotobacter*, *Bacillus* (Table 53.1c), *Beijerincka*, *Burkholderia*, *Enterobacter*, *Herbaspirillum*, *Klebsiella*, *Paenibacillus*, *Pseudomonas* (Table 53.1d), and *Stenotrophomonas* (Dobbelaere et al., 2003; see Fig. 53.2). Using mutants unable to fix nitrogen, Hurek et al. (2002) showed that the beneficial effects of the endophytic diazotrophic bacteria *Azoarcus* sp. on Kaller grass are directly associated with their nitrogen-fixing ability and this is also true for *Acetobacter diazotrophicus* on sugarcane (Sevilla et al., 2001).

Klebsiella pneumoniae and *Azospirillum* are free-living nitrogen-fixing rhizosphere bacteria. In the past, the plant growth-promoting properties of *Azospirillum* were thought to be due to its N_2-fixing property but recent developments show that this property is mainly due to its ability to produce the root-architecture-influencing hormone, auxin (See Section 53.4.1; see Chapters 27, 29).

53.3.2 Phosphate Solubilization

After water and nitrogen, phosphorus is the third plant growth-limiting compound. Phosphorus plays a role in numerous plant processes including energy generation, nucleic acid synthesis, photosynthesis, respiration, and cellular signaling (Vance et al., 2003).

Plants can absorb phosphorus only as $H_2PO_4^-$ and HPO_4^{2-} ions. Most soils contain amounts of phosphate which are in principle sufficient to support plant growth. However, many of these organic and inorganic forms are not accessible for the plant. Also phosphorus added to the soil as a soluble chemical fertilizer can be rapidly fixed into insoluble forms and thus made unavailable to plants (Rodriguez and Frago, 1999; Igual et al., 2001; Smyth, 2011).

Plants react to P-limitation by acidification of the rhizosphere, by increased growth of roots toward unexploited soil zones, by increasing the number of root hairs, and by secreting phosphatases. Acidification is the result of secretion of organic anions together with protons. Organic anions, with citrate and oxalate being more effective than others, can directly facilitate the mobilization of phosphate (Richardson et al., 2009; See Fig. 53.3).

Phosphorus is widely applied as a chemical fertilizer, and the excessive and unmanaged application of phosphorus can have negative impacts on the environment, including the eutrophication and hypoxia of lakes and marine estuaries (Smyth, 2011).

Some bacteria, referred to as *phosphate-solubilizing bacteria* (Igual et al., 2001; Kim et al., 1998), are able to solubilize bound phosphorus from organic or inorganic molecules, thereby, making it available for the plant (Lipton et al., 1987; See Fig. 53.3). Phosphate-solubilizing bacteria are ubiquitous and *Bacillus* (Table 53.1c), *Enterobacter*, *Erwinia*, and *Pseudomonas* spp. (Table 53.1d; see also Chapter 58) are amongst the most potent species. Production of organic acids such as gluconic acid is a major factor in the release of phosphorus from mineral phosphate (Rodriguez et al., 2006). Also, the release of a range of enzymes results in the generation of phosphate forms which can be taken up by the plant (See Fig. 53.3). These include nonspecific phosphatases that dephosphorylate phosphor–ester and/or phosphoanhydride bonds in organic matter, phytases that release phosphorus from phytic acid (Idriss et al., 2002), phosphonatases, and C–P lyases that dissociate C–P bonds in organophophonates (Rodriguez et al., 2006). Vyas and Gulati (2009) showed that phosphate-solubilizing *Pseudomonas* spp. are able to increase both the growth and phosphorus content

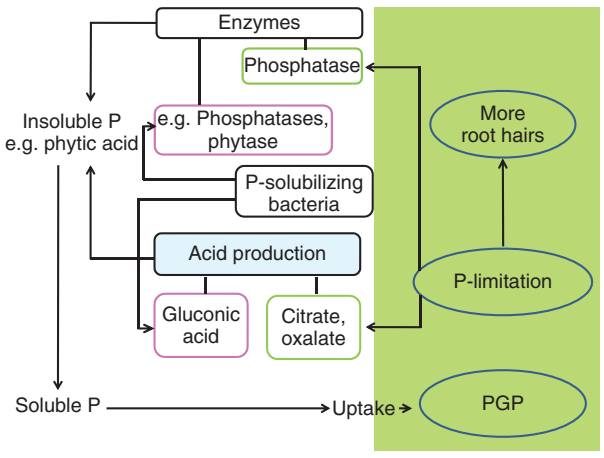

Figure 53.3 Microbial contribution to plant P-nutrition. For explanation, see text. Colors: green, plant; red, microbes; blue, processes.

of maize (see also Chapter 58). Sundara et al. (2002) showed that a phosphate-solubilizing *Bacillus megaterium* increases both the amount of plant-available phosphorus as well as the yield of sugarcane. De Freitas et al. (1997) showed that phosphate-solubilizing *Bacillus* spp. increase the yield of canola. Using molecular techniques, it was possible to identify a possible new mechanism involved in P-solubilization: assessing a genomic library of *P. fluorescens* B16, pyrroloquinoline quinone (PQQ) biosynthetic genes were identified responsible for PGP in this strain (Choi et al., 2008).

AMF were initially thought to provide the plant only with phosphorus. Since it is now known that AFM has a more general function, AFM has been described under Section 53.2.1.

53.3.3 Fe and Siderophores

Iron is an essential element for all organisms. Iron is an abundant element on the earth's crust but it is hardly soluble and therefore not suitable for uptake by living organisms. The concentration of Fe^{3+}, the form of iron ions available for living organisms, is only 10^{-18} M.

Plants produce and excrete chelators and/or phytosiderophores which bind Fe^{3+} and transport it to the root surface where it is either reduced to Fe^{2+}, which is subsequently taken up by the plant, or it is absorbed as an Fe^{3+}–phytosiderophore complex by the plant (Lemanceau et al., 2009; See Fig. 53.4; see Chapter 113).

Bacteria, growing under low Fe^{3+} concentrations, also produce a variety of siderophores which bind this ion with high affinity (See Fig. 53.4). A number of plant species

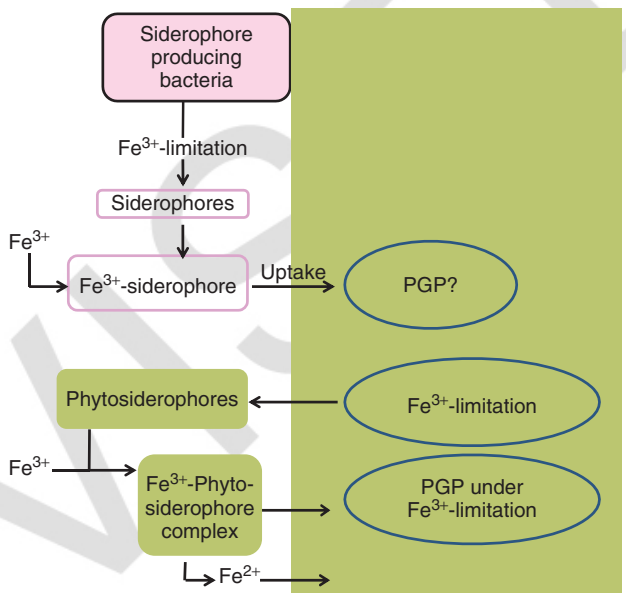

Figure 53.4 Possible microbial contribution to plant Fe-nutrition. For explanation, see text. Colors: green, plant; red, microbes; blue, processes.

can absorb bacterial Fe^{3+}–siderophores complexes, but it is unclear whether the uptake of these complexes has any significance to plant iron nutrition and/or direct PGP (Zhang et al., 2008).

53.3.4 Mixtures of Biofertilizers

Wu et al. (2005) performed a thorough greenhouse study to evaluate the effect of a mixture of four biofertilizers, namely an AMF (*Glomus mossae* or *Glomus intraradices*), an N-fixer (*Azobacter chroococcum*), a P-solubilizer (*B. megaterium*), and a K-solubilizer (*Bacillus mucilaginous*) on growth of *Zea mays* and soil properties. Controls were no fertilizer, chemical fertilizer, organic fertilizer, and two types of biofertilizers. The mixture of the four microbes significantly increased the growth of *Z. mays* and resulted in the highest biomass and seedling height. It also increased assimilation of N, P, and K. Moreover, soil properties such as organic matter and total N in soil were increased. The presence of the bacteria in the inoculum resulted in an at least fivefold higher root infection rate by AMF.

53.4 MICROBIAL PLANT GROWTH REGULATORS

Plants produce phytohormones or plant growth regulators, that is, compounds, which at concentrations lower than 1 μM can regulate plant growth and development. There are six classes of plant hormones, namely, auxins, brassinosteroids, cytokinins, gibberellins (GAs), abscisic acid (ABA), ethylene (ET), and the recently discovered SLs (see Chapters 27–35). Phytohormones regulate processes such as cell division, cell expansion, differentiation, shoot branching, and cell death. Phytohormone pathways and cross talk between them play a key role in process coordination and cellular responses (Moller and Chua, 1999; Santner et al., 2009).

Many rhizosphere bacteria can produce plant growth regulators, such as auxins, cytokinins, GAs, ABA, and ET *in vitro* (Zahir et al., 2003; see also Chapter 27). Bacteria which produce ABA and ET are called *stress controllers*. As presently known, brassinosteroids and SLs are not produced by bacteria or fungi.

Phytohormone production by microbes can modulate the endogenous plant hormone levels and consequently can have an enormous influence on plant growth and development (Gray, 2004; van Loon, 2007). For details on hormones produced by plants and rhizosphere bacteria, the reader is referred to excellent reviews by García de Salome et al. (2006), and Spaepen et al. (2009), as well as Chapters in Section 4.

53.4.1 Auxins

Non-conjugated indole-3-acetic acid (IAA) is the most abundant member of the auxin family. The concentration of auxin and the ratio of auxin to other hormones are critical for the physiological response of the plant (Lambrecht et al., 2000; see Chapters 27, 29).

It has been estimated that up to 80% of the rhizosphere bacteria can synthesize IAA (Khalid et al., 2004; Patten and Glick, 1996). Bacteria which produce IAA can add to, or influence, the levels of endogenous plant auxin (Patten and Glick, 1996). It is assumed that PGP by exogenously added auxin, acts by increasing root growth, length, and surface area, thereby, allowing the plant to access more nutrients and water from the soil (Vessey, 2003; See Fig. 53.5).

Rhizosphere bacteria can use several different pathways for IAA biosynthesis. Most of them use tryptophan, secreted by the plant as a component of root exudate, as a precursor (Costacurta and Vanderleyden, 1995; Spaepen et al., 2007; Spaepen et al., 2009a, 2009b; see also Chapter 29; see Fig. 53.5). Indeed, Kamilova et al. (2006) observed that *P. fluorescens* biocontrol strain WCS365, which produces IAA in the presence of tryptophan, is able to stimulate root growth of radish, a plant which secretes high amounts of tryptophan in its exudate, but not of tomato, sweet pepper, or cucumber plants, which secrete at least 10-fold less tryptophan.

Azospirillum brasilense is an N_2-fixer which promotes plant growth by increasing its root surface through shortening the root length and enhancing root hair formation. It has been thought for a long time that its plant growth-promoting ability was based on N_2 fixation. However, the present notion is that auxin production is the major factor responsible for root changes and therefore its plant growth-promoting properties (Pliego et al., 2011; See Fig. 53.5). This notion is based on the following observations. (i) Dobbelaere et al. (1999) showed that the effect of the wild-type strain on the root can be mimicked by the addition of pure auxin. (ii) A mutant strain strongly reduced in IAA production did not induce the root changes and, (iii), a strain constitutive for IAA production showed the same effect on the root changes as the wild-type strain but already at lower bacterial cell concentrations (Spaepen et al., 2008). Interestingly, when the amount of root exudate becomes limiting for bacterial growth, *A. brasilense* increases its IAA production, thereby, triggering lateral root and root hair formation which results in more exudation and, therefore, in further bacterial growth. In this way, a regulatory loop is created which connects plant root proliferation with bacterial growth stimulation (Spaepen et al., 2009b).

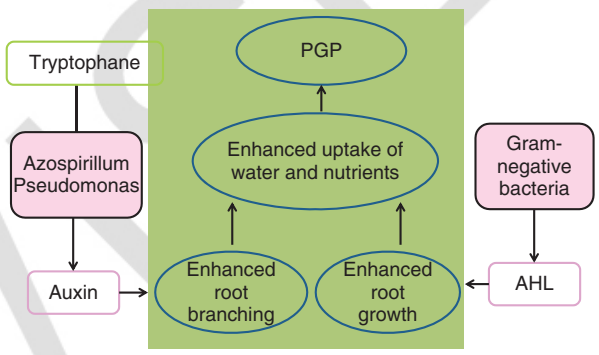

Figure 53.5 Stimulation of root branching and growth by auxin. For explanation, see text. Colors: green, plant; red, microbes; blue, processes.

53.4.2 Cytokinins

Zeatin is the major representative of a group of molecules called *cytokinins*. Cytokinins have the capacity to induce division of plant cells in the presence of auxin. Starting from callus tissue, the ratio between the amounts of auxin and cytokinin determines whether callus differentiates in root or shoot: high auxin promotes root differentiation, whereas high cytokinin promotes shoot morphogenesis. Equimolar concentrations induce cell proliferation.

Cytokinin production is linked to callus growth of tobacco. A test based on this principle can be used as a screening method for cytokinin-producing bacteria. Many rhizosphere bacteria can produce cytokinins in pure culture, e.g. *Agrobacterium*, *Arthrobacter*, *Bacillus*, *Burkholderia*, *Erwinia*, *Pantoea agglomerans*, *Pseudomonas*, *Rhodospirillum rubrum*, *Serratia*, and *Xanthomonas* (reviewed in García de Salome et al., 2001). The spectrum of cytokinins produced by rhizobacteria is similar to that produced by the plant (Barea et al., 1976; García de Salome et al., 2001; Frankenberger and Arshad, 1995) of which isopentenyladenine, trans-zeatin, cis-zeatin, and their ribosides are the most commonly found representatives.

García de Salome et al. (2001) provided evidence for a role of cytokinin of rhizosphere bacteria in PGP. They used mutants of *P. fluorescens* strain G20-18 which produces reduced amounts of cytokinin and normal amounts of auxin. In contrast to the wild-type strain, the mutants appeared to be unable to promote growth of wheat and radish plants (García de Salome et al., 2006).

Concerning the mechanism of action of cytokinins, one speculates that cytokinin produced by rhizosphere bacteria becomes part of the plant cytokinin pool, and thus influences plant growth and development.

The ability to produce auxins and cytokinins is a virulence factor for the pathogen *Agrobacterium tumefaciens*, which produces crown galls. This bacterium can transfer the genes for the production of auxins and cytokinins to the plant and incorporate these genes in the plant's DNA (see Spaink et al., 1998). Another bacterium from this genus, *A. rhizogenes*, modifies cytokinin metabolism, resulting in the appearance of masses of roots—instead of callus—from the infection site (Hamill et al., 1993).

53.4.3 GAs

These hormones consist of a group of terpenoids with 20 carbon atoms, but active GAs only have 19 carbon atoms. This group of compounds consists of over 130 different molecules (Dodd et al., 2010; see Chapter 31). GAs are mainly involved in cell division and cell elongation within the subapical meristem, thereby, playing a key role in internode elongation. Other processes affected by these hormones are seed germination, pollen tube growth, and flowering in rosette plants. Similar to auxins and cytokinins, GAs mainly act in combination with other hormones.

Bacteria which produce GAs, such as *Acinetobacter*, *Agrobacterium*, *Arthrobacter*, *A. brasilense*, *Azospirillum lipoferum*, *Azotobacter*, *Bacillus*, *Bradyrhizobium japonicum*, *Clostridium*, *Flavobacterium*, *Micrococcus*, *Pseudomonas*, *Rhizobium*, and *Xanthomonas*, secrete it in the rhizosphere (Frankenberger and Arshad, 1995; Gutiérrez Manero et al., 2001; Rademacher, 1994; Tsavkelova et al., 2006). Hardly anything is known about GA synthesis in rhizosphere bacteria.

Kang et al. (2009) showed that culture suspensions of GA-producing *Acinetobacter calcoaceticus* were able to increase the growth of cucumber, Chinese cabbage, and crown daisy. The mechanism of plant growth stimulation by GAs is still rather obscure. It is thought that bacteria may increase GA levels *in planta* by either producing GAs, deconjugating GAs from root exudates, or hydroxylating inactive GA to active forms (Bottini et al., 2004; see Chapter 31). Fulchieri et al. (1993) speculate that GAs increase root hair density in root zones involved in nutrient and water uptake.

53.4.4 ABA

ABA is a 15-carbon compound, which, similar to ET, is involved in plant responses to biotic and abiotic stresses. It inhibits seed germination and flowering. It is involved in protection against drought, salt stress, and toxic metals. It also induces stomatal closure (Smyth, 2011).

ABA can be produced in culture media by several bacteria such as *A. brasilense* (Cohen et al., 2008; Perrig et al., 2007) and *B. japonicum* (Boiero et al., 2007). ABA levels *in planta* have been increased in *Arabidopsis thaliana* by *A. brasilense* Sp25 (Cohen et al., 2008).

The effect of inoculation with ABA-producing bacteria on plant growth is experimentally poorly underpinned. Since ABA inhibits the synthesis of cytokinins (Miernyk, 1979), it was speculated that ABA increases plant growth by interfering with the cytokinin pool (Spaepen et al., 2009). It could also alleviate plant stress by increasing the root/shoot ratio (Boiero et al., 2007).

53.4.5 ET and ACC Deaminase

ET is a gaseous hormone best known for its ability to induce fruit ripening and flower senescence. ET affects numerous plant developmental processes including root growth, root hair formation, flowering, fruit ripening, and abscission, and leaf and petal senescence and abscission (Dugardeyn and van der Straeten, 2008). ET usually inhibits both primary root elongation and lateral root formation but it can also promote root hair formation (Dodd et al., 2010). It generally inhibits stem elongation in most dicots favoring lateral cell expansion and leading to swelling of hypocotyls. ET also breaks seed and bud dormancy. ET production is typically upregulated in plants in response to pathogen attack, heat and cold stress, waterlogging, drought, excess heavy metals, high soil salinity, and soil compaction (Dodd et al., 2010; Glick, 2005).

ET is synthesized under biotic stress conditions following infection by pathogens, as well as by abiotic stress conditions such as drought. It is therefore also known as the *stress hormone*. In the plant, ET is produced from *S*-adenosylmethionine (SAM) which is enzymatically converted to ACC and 5′-deoxy-5′methylthioadenosine (MTA) by ACC synthase (Giovanelli et al., 1980; See Fig. 53.6).

The enzyme ACC deaminase is present in many rhizosphere bacteria, such as *Achromobacter*, *Pseudomonas*, and *Variovorax*, and in the fungus *Trichoderma*. Such microbes can take up ACC secreted by the plant root and convert it into α-ketobutyrate and ammonia (Glick et al., 2007a; Fig. 53.6). This results in the decrease of ACC levels, and therefore also of ET levels, in the plant and in decreased plant stress. Inoculation of plants with ACC deaminase-producing bacteria can protect plants against stress caused by flooding, salination, drought, waterlogging, heavy metals, toxic organic compounds, and pathogens (Berg, 2009; Glick, 2005; Glick et al., 2007a,2007b; Belimov et al., 2005). ACC deaminase activity has been found in fungi such as *Trichoderma* (Viterbo et al., 2010) and in free-living soil bacteria, endophytes, and rhizobia from a wide range of genera and there have been many correlations between ACC deaminase activity in a range of bacteria and their ability to promote plant growth under various conditions, for

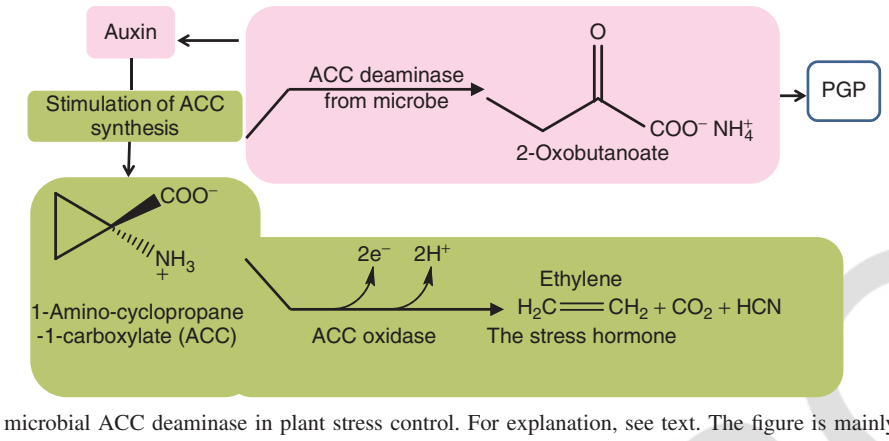

Figure 53.6 Role of microbial ACC deaminase in plant stress control. For explanation, see text. The figure is mainly based on papers by the group of B. Glick. According to his hypothesis (Glick et al., 1998), bacterial auxin activates plant ACC synthase. The produced ACC can be used by some microbes as an N-source, thereby, decreasing ethylene levels. In order to explain how ACC produced by the plant is converted by ACC deaminase from the bacterial cytoplasm, Glick et al. (1998) assumed that a significant portion of ACC is exuded from plant roots and seeds and then taken up by the microbe. We would like to suggest the following alternative explanation, namely that the microbe uses the type three secretion system (TTSS) for this purpose as it has been proposed earlier that a beneficial bacterium uses the needle of the TTSS to suck nutrients from the plant root (De Weert et al., 2007). Another possibility is that the bacterium uses its TTSS to deliver the enzyme into the plant. In the case of *Trichoderma*, one can imagine that its endophytic localization facilitates contact between enzyme and substrate. Colors: green, plant; red, microbes; blue, processes.

example, in wheat (Zahir et al., 2009), maize (Shaharoona et al., 2006), and tomato (Grichko and Glick, 2001; Mayak et al., 2004a,2004b).

In addition to a direct role of ET on plant growth, this hormone can also act as a virulence factor and a signaling molecule in plant protection against pathogen attack. ET production was reported to act as a virulence factor for bacterial pathogens, for example, *Pseudomonas syringae* (Weingart and Volksch, 1997; Weingart et al., 2001). Furthermore, ET acts as a signaling compound in induced systemic resistance caused by some rhizobacteria (Van Loon, 2007).

53.4.6 Volatiles

Bacteria can produce a wide range of volatiles. While the biological function of most of these volatiles is not fully understood, it is assumed that they are involved in a number of processes including cell–cell signaling, interspecies signaling, and a possible carbon release valve; these compounds can promote plant growth and act as microbial inhibiting agents (Wheatley, 2002; Vesperman et al., 2007; Kai et al., 2009; see also Chapter 63).

Bacterial volatiles, produced by *Bacillus* spp., have been shown to promote plant growth in *A. thaliana*. The highest level of growth promotion was observed with 2,3-butanediol and its precursor acetoin (Ryu et al., 2003).

Farag et al. (2006) identified 38 volatile compounds from rhizobacteria. Blom et al. (2011) screened 42 strains grown in four different growth media on the growth response of *A. thaliana*. Under at least one of these conditions, each strain showed significant volatile-mediated plant growth modulation. Only one strain, a *Burkholderia pyrrocinia*, showed significant PGP on all four media. The volatiles indole, 1-hexanol, and pentadecane showed PGP but the results suggested that this occurred only under stress conditions.

53.4.7 AHLs

AHLs are signal molecules secreted by many bacteria. When their extracellular concentration reaches a certain value, the quorum, they play a role in many processes such as secretion of antibiotics and exo-enzymes (see Chapter 71). For a more detailed discussion on AHLs and their action (see Chapters 70–78). In terms of growth promotion, it was shown recently that 10 μm C6-AHL and C8-AHL increase root growth in *A. thaliana* (See Fig. 53.5; see also Chapter 73). This is accompanied by an increase in the auxin/cytokinin ratio and in increased expression of over 700 genes in the roots and of a lower number in the stem (von Rad et al., 2008).

53.4.8 *nod* Gene Inducers and LCO's (Nod Factors)

The *nod* genes of (*Brady)Rhizobium* are induced by flavonoids or isoflavonoids (see Chapter 51). LCO's signal molecules are the products of *nod* genes. They initiate root hair curling and subsequent steps in the nodulation of leguminous plants by (*Brady)rhizobium* bacteria (see Section 53.3.1).

Interestingly, the inducers as well as the products of the *nod* genes promote plant growth and this effect is not restricted to leguminous plants (See Fig. 53.2). See http://www.bioag.novozymes.com. For example, one product is based on isoflavonoids and is claimed to activate mycorrhizae before the plant does so, resulting in enhancing nutrient uptake, which in turn leads to lateral root development and stress tolerance. Formulations for soybean, peanut, alfalfa, and pea/lentil, combining the respective LCO and rhizobia, have also been commercialized. When LCOs were applied on seeds of the non-legumes, corn, cotton, and wheat, increased plant growth and yield were observed in the field. Furrow applications and foliar sprays have similar effects. Possible explanations given are enhanced germination, early seedling growth, increased photosynthesis, enhanced nutrient uptake, and enhanced LCO-stimulated mycorrhizal root colonization (Smith et al., 2011).

53.5 CONCLUSIONS

Nitrogen and phosphorus are the major chemical fertilizers applied to enhance crop yield. This raises a number of concerns such as water contamination leading to eutrophication and health risks for humans. Moreover, it results in soil degradation and loss of biodiversity. Presently, the cost for nitrogen fertilizer is steeply increasing as a consequence of the increasing energy prices. The amount of available potassium is limited. For these reasons, the interest in sustainable fertilization, using microbes, is strongly increasing. In this chapter, we have discussed many microbes that can be applied for a more environmentally friendly agriculture.

ACKNOWLEDGMENT

The authors thank Dr. Doreen Schachtschabel for discussions about strigolactones and her help with Figure 53.6.

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